THE SITE OF STEROL AND SQUALENE SYNTHESIS IN THE HUMAN SKIN[1,2,3]

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In earlier work (1) it was demonstrated that human scalp skin is an efficient organ for synthesizing fatty acids, squalene and sterols from 1-C^{14} acetate, *in vitro*. In that work squalene was isolated from the lipid mixture in a comparatively pure condition, and its specific activity was found to be 10 times higher than that of the sterols. This higher specific activity met an important and contested (2, 3) requirement that if squalene is to serve as a cholesterol precursor, as recent evidence indicates (4, 5), its specific activity must be higher than that of cholesterol.

From the point of view of cutaneous biology it seemed to be of interest to know to what extent the different structural elements of the skin, namely, epidermis, corium and sebaceous glands participate in these different syntheses. It has been an old contention that cholesterol on the skin surface is a product of the keratinizing epidermis rather than of sebaceous glands, mainly because of the unusually high cholesterol content of scales in exfoliative dermatitis (6).

This problem of the site of synthesis of squalene and cholesterol was studied in a surgical specimen of an amputated arm. The epidermis was separated from the corium in specimens of both forearm and palmar skin, and each sample was incubated with 1-C^{14} acetate in a physiological medium. The lipids were then extracted and their constituents examined for radioactive uptake.

EXPERIMENTAL

Preparation of specimens. Normal skin was obtained from the forearm and palm of a 57-year-old woman immediately after amputation of the left arm for an osteosarcoma. The skin was carefully freed from subcutaneous fat and placed into ice cold normal saline until four different specimens could be prepared for incubation: 1. *Forearm epidermis* (FE). Forearm epidermis was obtained by the stretch method (7) after the skin was first cut into strips 8 x 1 cm. The epidermal sheets thus obtained were then cut into small pieces with scissors. Duplicate samples (FE_1 and FE_2) were prepared. 2. *Forearm corium* (FC). The tissue remaining after the epidermis was removed was cut into pieces 1 $cm.^2$, then each of these was sliced parallel to the skin surface into slices approximately 0.5 mm. thick. Duplicate samples (FC_1 and FC_2) were prepared. 3. *Palmar corium* (PC). To save time and prevent enzymic deterioration, separation of the epidermis

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of the palm by the stretch method was not attempted. However, a specimen of the deep corium layer was prepared by first cutting a rectangle from the center of the palm 7 x 4.5 cm. and cutting this into smaller squares 7 x 7 mm. Three slices, about 0.5 mm. thick, were then removed from the lower surface of the corium parallel to the surface, and sufficiently far from it to make sure that no epidermis was included. 4. *Palmar epidermis and upper corium* (PEC). The palmar skin remaining after specimen PC was removed contained about ¼ of the original thickness of the corium and the whole epidermis. This was sliced parallel to the surface into slices approximately 0.5 mm. thick.

Incubation. Each sample was incubated for 7 hours at 37°C in a Krebs-Ringer phosphate buffer solution, pH 7.4, with glucose and 50 μ moles 1-C^{14} acetate counting 4.23×10^6 counts per minute as an infinitely thick $BaCO_3$ sample. The gas phase was 100% oxygen. The incubation was stopped with alcohol and the specimens stored at 0° until the lipids could be extracted.

Chemical analysis of the lipid constituents. The extraction of the lipids with absolute alcohol and ether, the saponification and chromatography on alumina and silica gel were carried out in the manner previously described (1).

Radioactivity of the lipid constituents. The procedure applied for measuring radioactivity of the lipid constituents will be published in detail elsewhere.

RESULTS AND DISCUSSION

The analysis of the lipids obtained from the various specimens is shown in Table I. It can be seen that forearm epidermis contains 10 times the amount of sterols when the latter are expressed as percentage of dry tissue weight, than

TABLE I

Analysis of the lipids of various skin specimens

Sample	Kind of Skin	Wet Tissue Weight*	Dry Tissue Weight†	Total Lipids	Fatty Acids	Unsaponifiable	Squalene‡ as % of Dry Tissue Weight	Sterols§ as % of Dry Tissue Weight
		g.	*g.*	*mg.*	*mg.*	*mg.*		
FE_1	Forearm epidermis	1.5	0.135	28.4	21.0	6.22	—	—
FE_2	Forearm epidermis	1.5	.155	34.8	24.6	6.90	0.28	2.7
FC_1	Forearm corium	4.0	.417	52.1	47.7	3.00	.15	.19
FC_2	Forearm corium	4.0	.541	74.7	67.8	3.03		
PEC	Palmar epidermis plus upper corium	3.0	.445	21.6	13.4	4.68	~.02	.68
PC	Palmar lower corium	3.0	.350	29.7	26.3	3.06	~.02	.31
S	Scalp¶ (total skin)	3.0	.435	110.2	90	10.3	.5	.33

* Adhering moisture was not completely removed.

† Weight includes lipids.

‡ Isolated by chromatography of the unsaponifiable matter on alumina followed by silica gel, then weighed (Ref. 1).

§ Determined by digitonin precipitation of the ether eluate of the chromatography of the unsaponifiable matter on alumina (Ref. 1).

¶ Data taken from Ref. 1.

does the corium of the forearm. The epidermal specimen of the palm, PEC, although containing a fair amount of corium, also has a considerably higher sterol content than the pure corium sample, PC. This suggests that the epidermis is more active in sterol synthesis than is the corium. In contrast, the squalene content of the epidermis and corium of the forearm are in the same range of magnitude. The squalene content of the two palm specimens are also about equal. The most significant finding is, however, that the amounts of squalene in the forearm specimens are 10 times greater than in palmar skin. The main structural difference in the skin of these two sites is that the forearm skin contains sebaceous glands while palmar skin has none. This suggests that sebaceous glands produce squalene but convert it only slowly, if at all, into cholesterol and other sterols, whereas in epidermal synthesis conversion into sterols is rapid and more complete. Further evidence to support this contention is found in the data on radioactivity.

That squalene, sterols and fatty acids are synthesized from 1-C^{14} acetate by all four specimens was established by the fact that the respective fractions were all radioactive. A detailed report of the specific and total activities of the lipid constituents of the various specimens as well as proof of the presence of squalene and its biosynthesis by palm skin will be published elsewhere. In data to be published it was found that epidermis of both forearm and palm incorporate approximately ten times the amount of isotope per gram of tissue than do the respective corium samples. In this preliminary report we present only the distribution of isotope among the various lipid constituents of the different skin specimens (Table II). In those specimens which contain sebaceous gland parenchyma, namely, the corium of the forearm and the total skin (epidermis plus corium) of the scalp, there is, in our *in vitro* experiments, a much higher incorporation of radioactivity into squalene, inasmuch as 32% and 40% respectively of the total isotope of the lipids are in squalene, while in all other samples, none of which contain sebaceous glands, the radioactivity in the squalene portion is very small. This coincides with the tentative conclusion drawn above from the analytical data, that sebaceous glands are primarily responsible for the presence of squalene.

If this conclusion is correct, earlier findings concerning age differences in

TABLE II

Distribution of radioactivity in the lipid constituents by various skin specimens

Sample	Kind of Skin	Fatty acids	Sterols	Squalene	Total
		%	%	%	%
FE_2	Forearm epidermis	66	23	1.1	90
FC_{1+2}	Forearm corium	43	20	32	95
PEC	Palmar epidermis plus upper corium	52	40	1.7	94
PC	Palmar lower corium	80	17	2.3	99
S	Scalp* (total skin)	52	2.6	40	94

* Data taken from Ref. 1.

relative cholesterol and squalene content of surface fat (8, 9) can now be interpreted in a new manner. Children's hair fat contains relatively more cholesterol because in childhood participation of sebaceous glands in the production of surface fat is much less than in adulthood. With the development of sebaceous glands during puberty, the squalene content of hair fat increases relative to sterols, the latter being products of epidermal cells and presumably not changing substantially with age.

SUMMARY

1. The analysis of the lipids and the isotopic incorporation of 1-C^{14} acetate into the lipids of forearm epidermis, forearm corium, palmar epidermis and palmer corium of human skin was studied.

2. All specimens were found to synthesize fatty acids, sterols and squalene.

3. Evidence is presented for the hypothesis that the main site of sterol synthesis is the keratinizing epidermis while the main site of squalene synthesis is the sebaceous gland.

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DISCUSSION

Dr. Victor H. Witten: (New York, N. Y.). I may have missed a point. I don't understand how you were able to label the cholesterol, and how you finally went about counting the radioactive material.

Dr. Nicholas Nicolaides (in closing): It was previously shown by many

authors that if you incubate radioactive acetate with liver slices, as I did with skin slices, you are able to isolate radioactive cholesterol from the lipid mixture. Other lipids have also been shown to incorporate radioactivity by this method. And the fatty acids, the squalene and the cholesterol isolated from the different skin specimens in this study were all found to be radioactive. From the manner in which these lipids were isolated, none of the original acetate used for incubation could have been carried along and contaminated them. The acetate was washed away by procedures which have been tested for completeness of its removal. Furthermore, others, using such procedures, have demonstrated that if the C^{14} isotope is present only in the carboxyl position of the acetate used for the incubation, only certain carbon atoms, (alternate ones, for instance, in the case of the fatty acids) are radioactive, the others being non-radioactive. The acetate is used as building blocks for the synthesis of these lipids.